

**An Apparent Induction of Secondary
Dormancy in *Eucalyptus globulus* Labill
Seeds**

by

Thushara Sivasankaran Nair

**Submitted in fulfilment of the requirements for the degree
of Doctor of Philosophy**



School of Agricultural Science

University of Tasmania

Hobart

October, 2006

Morris
thesis
NAIR
PhD
2006

THE
UNIVERSITY
OF TASMANIA
LIBRARY

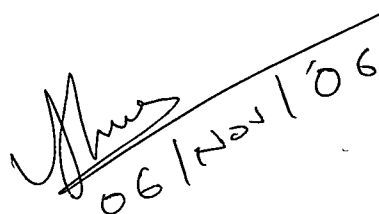
A 7002 1332193B

Declaration

I certify that this thesis contains no material which has been accepted for the award of any other degree or diploma in any other University, and to the best of my knowledge and belief this material contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Authority of Access

This thesis may be made available for loan and limited copying in accordance with the Copyright Act 1968.

A handwritten signature, possibly 'Thushara', is written over a diagonal line. Below the line, the date '06/Nov/06' is written.

Thushara Sivasankaran Nair

School Agricultural Science

University of Tasmania

Table of Contents

Declaration	i
List of Figures	vi
List of Plates and Tables	viii
Acknowledgement	x
Abbreviations and Symbols	xii
Species Name	xiii
Abstract	xvii
Chapter-1 General Introduction	1
Chapter-2 Literature Review	7
1. Introduction	7
1.1. <i>Eucalyptus</i> taxonomy and distribution	7
1.2. <i>Eucalyptus globulus</i>	8
1.3. Economic importance of <i>E. globulus</i>	9
1.4. Extent and location of <i>E. globulus</i> plantations	10
1.5. Breeding activity in <i>E. globulus</i>	10
1.6. Nursery seed production	10
1.7. Orchard seed description	11
2. Seed structure	13
2.1. <i>Eucalyptus</i> fruit and seed structure	13
3. Seed Quality	14
3.1. Seed germination and type of germination in Eucalypts	14

3.2. Seed vigour	15
3.2.1. Factors affecting germination and vigour	15
i. Temperature	16
ii. Light	17
iii. Moisture	18
iv. Abscissic acid (Growth inhibitor)	19
v. Age of seeds	19
3.3. Seed size	20
4. Seed Dormancy	21
4.1. Dormancy and its Ecological Role	21
4.2. Types of Dormancy	21
4.2.1. Primary dormancy	21
I. Types of Primary dormancy	
i. Exogenous dormancy	21
ii. Endogenous dormancy	23
II. Dormancy in <i>Eucalyptus</i> spp.	25
4.2.2. Secondary dormancy	26
I. Environmental factors regulating secondary dormancy	
i. Temperature	26
ii. Light and dark	27
iii. Water	28
iv. Gases	28
5. Methods of Improving Germination and Overcoming Dormancy	29
5.1. Stratification	29
5.2. Priming	31
5.3. Wetting and Drying Cycles	32
5.4. Smoke	32
5.5. Growth Hormones and other Compounds	33
6. Conclusion	35
 Chapter-3 General Materials and Methods	 36
3.1. Seedlot	36

3.2. Germination Conditions	39
3.3. Experimental Design and Statistical Analysis	40
Chapter-4 Water Relations in <i>E. globulus</i> Seed	41
4.1. Introduction	41
4.2. Materials and Methods	46
4.3. Results	48
4.4. Discussion	54
Chapter-5 Induction of Secondary dormancy (Dehydration and Light Effects)	57
5.1. Introduction	57
5.2. Materials and Methods	59
5.3. Results	61
5.4. Discussion	69
Chapter-6 Light effects	72
6.1. Introduction	72
6.2. Materials and Methods	74
6.3. Results	77
6.4. Discussion	82
Chapter-7 Light Quality	85
7.1. Introduction	85
7.2. Materials and Methods	89
7.3. Results	96
7.4. Discussion	107
Chapter-8 General Discussion	111
8.1. Practical solutions	111
8.2. Physiological aspects	113
8.3. Conclusion	116

Chapter-9	References	117
Chapter 10	Appendices	140

List of Figures

- Fig-4.1:** Water uptake with time after the start of imbibition on wet filter paper. Data points are results of 4 replicates of 50 seeds \pm standard error of the mean.50
- Fig-4.2:** Water uptake with time after the start of imbibition by seedlot “OS” in full light (Δ) and complete darkness (\blacksquare). Data points are results of 4 replicates of 50 seeds \pm standard error of the mean.51
- Fig 4.3:** Seed water potential with time after the start of imbibition on wet filter paper. Data points are results of 4 replicates of 50 seeds \pm standard error of mean.52
- Fig 4.4:** Water uptake as relative water content (a) and water potential (b) of untreated (\blacktriangle) and 30 h imbibed followed by 7 days drying (\blacksquare) shown in this figure. Data points are results of 4 replicates of 50 seeds \pm standard error of the mean.53
- Fig 5.1 (a & b):** Mean germination percentage on day 5 (\diamond), 8 (\square) and 10 (Δ) for seedlots ‘h’ (a) and ‘Li’ (b), germinated after soaking in laboratory conditions at 18-20 °C for 0-48 h (16/8 h of natural light/dark cycle). Data points are results of 4 replicates of 50 seeds \pm standard error of the mean.63
- Fig. 5.2.** Mean germination percentage on days 5 (\diamond) and 11 (\square) for seed lot ‘h’ (a) and ‘Li’ (b), germinated after different periods of dark soaking at 25 ± 1 °C followed by 7 days drying back at room temperature (18-20 °C) in natural light conditions. Data points are results of 4 replicates of 50 seeds \pm standard error of the mean. LSDs for comparison between soaking times on day 5 were 10.3 and 12 respectively and for day 11, 11.1 and 13.7 respectively.64
- Fig. 5.3:** Germination in alternating light/dark (a) and darkness (b) on days 6 (\diamond, \blacklozenge), 10 (\square, \blacksquare), 13 ($\triangle, \blacktriangle$) and 19 (\circ, \bullet) for seedlot ‘KI’ imbibed for different time periods followed by drying back for 7 days at 18-20 °C in natural light conditions. Data points are results of 4 replicates of 50 seeds \pm standard error of the mean.65

Fig. 5.4. Mean germination (%) in alternating light/dark (a) and darkness (b) on days 6 (◇, ◆), 10 (□, ■), 13 (△, ▲) and 19 (○, ●) for seedlot 'h' imbibed for different time periods followed by drying back for 7 days at 18-20 °C in natural light conditions. Data points are results of 4 replicates of 50 seeds ± standard error of the mean.....	66
Fig 5.5: Glasshouse germination on days 13, 15 and 20 for seed of seedlot 'Ki' imbibed in darkness for 0 or 30 h at 25 ± 1 °C and dried back and stored for 7 days at room temperature (18-20 °C). Germination was in alternating natural light and dark (□) or continuous dark conditions (■) Error bars are standard errors of the mean (n=5).....	67
Fig 6.3 (a & b): Germination in response to initial imbibition in darkness for seedlot 'A' (a) and 'H' (b). Seed was imbibed for different periods of complete darkness and transferred to light. Germination counts were on day 5(■) and day 11 (▲). Two controls were full light and constant dark (not shown in the figure). Data points are results of 4 replicates of 50 seeds ± standard error of the mean.....	80
Fig 6.4 (a & b): Germination in response to initial imbibition in light for seed lot 'KI-1' (a) and 'H' (b). Seed was imbibed for different period of light and transferred to constant darkness. Germination counts were done on day 9 (□) and day 14 (Δ). Data points are results of 4 replicates of 50 seeds ± standard error of the means.....	81
Fig 10.1: Spectral composition of Incubator or germination cabinet (Linder and May Model LMRIL-1-SD) lights.	140
Fig 10.2: Light emission from the 10,000MCD Red light peaking at 625 nm.	141
Fig 10.3: Light emission from 7000MCD White 5 mm diameter dome.....	142
Fig 10.4: Light emission from 5000MCD Blue 5 mm diameter dome peaking at 470 nm.....	143
Fig 10.5: Light emission from Far red 5 mm diameter dome peaking at 740 nm.	144

List of Plates and Tables

Plate 1.1: <i>Eucalyptus globulus</i> seeds germinate uniformly and are highly viable. Photograph shows Lannen ® type trays, generally used in commercial nurseries.....	2
Plate 1.2: A Lannen® tray from a commercial nursery showing uneven germination 5-6 weeks after sowing.....	3
Plate 1.3: Seedling trays in a shade house. Trays in the foreground show uneven germination evident about 4 weeks after sowing.....	5
Table 3.1: Details of Commercial Seedlots supplied.....	37
Table 3.2: Seedlot performance test (n=4).....	38
Plate 5.1: Photograph showing germination in glasshouse conditions. Strongest germination was in control (untreated) seed germinated in darkness (second from left). Treatments are D (dark germination), L/D (germination in natural light), 0 is non preimbibed seed and 30 is seed imbibed for 30 h and dried back for 7 days before sowing.....	68
Table 6.1 (a & b): Influence of light condition during germination on % germination after 7 days in seedlots 'A' and 'H'. Pre-treated seed was imbibed for 30 h in complete darkness followed by dehydration for 7 days (n=4). The control seed was not pre-treated. LSD (P=0.05) for comparison within the table for seedlot A is 11.0 with a significant interaction between light and pre-treatments. For seedlot 'H', there was a significant light effect (see text) but no interaction between light and pre-treatment, LSD.(P=0.05) is 13. By day 10 there was no significant treatment effects in both seedlots.	77
Table 6.5: The effect of duration of initial light exposure on mean germination percentage of seedlot 'h' on days 6, 8 and 9 (n=4).	82
Table 7.1: The effect of light quality on germination of seeds from seedlot 'KI-2' determined after 50 h (7.1 a) and 5 days (7.1 b) (n=4).....	97
Table 7.2: The effect of light quality following imbibition in constant darkness for 24 h on day 5 germination percentage of seed from seedlot 'KI-2' (a) and 'H' (b) (n=4).....	98

Table 7.3: The effect of light quality following imbibition in constant germination cabinet light for 24 h on day 5 germination of seeds from seedlot 'KI-2'. Germination result for seeds exposed to constant darkness and constant cabinet light from the start of the imbibition is included for comparison (n=4).	99
Table 7.4 (a&b): The effects of light quality during the first 24 h of imbibition on subsequent germination of seed from seedlot 'KI-2' under constant cabinet light or constant darkness. Germination percentages were determined 5 days after the start of imbibition (n=4).	100
Table 7.5 The effect of addition of 5×10^{-5} M GA ₃ at the start of imbibition and light quality on germination of seed from seedlot 'KI-2' (n=4). ..	101
Table 7.6 (a &b). The effect of light quality during the first 24 h of imbibition and time of application of 5×10^{-5} M GA ₃ on day 5 germination of seed from seedlot 'KI-2' (n=4).....	102
Table 7.7: The effect of light quality during the first 24 h of imbibition on day 5 germination of seed from seedlot 'KI-2' treated with 5×10^{-5} M GA ₃ at the start of imbibition. Seeds were subsequently germinated in constant darkness (n=4).	103
Table: 7.8: The effects of light quality on day 5 germination of seeds from seedlot 'KI-2' after imbibition in darkness for 24 h followed by addition of 5×10^{-5} M GA ₃ (n=4).	104
Table 7.9: The effects of one or two 30-minute pulses of red light during the first 24 h of imbibition in constant darkness on subsequent germination of seed from seedlots 'KI-2' and 'h' under constant cabinet light. First treatment is constant cabinet light. Germination was scored 5 days from start of imbibition (n=4).....	105
Table 7.10: The effects of one or two 30 minute pulses of red light during the first 24 h of imbibition in constant darkness on subsequent germination of seed from seedlot h under 12/12 light/dark period. Germination was assessed on day 5 (n=4).....	106

Table 7.11: The effect of GA₃ on germination of seed of seedlot 'h' exposed to different light qualities (red, far red, darkness or cabinet light) for the first 30 h of germination. 5×10⁻⁵ M GA₃ was added at 30 h (“-“= without GA₃ and “+”= with GA₃). With the exception of the continuous cabinet light treatment, seeds were subsequently germinated under constant darkness. Germination counts were done on day 7 (n=4). ...107

Acknowledgement

This thesis is a result of three years of hard work where I was supported by many people and now I have the opportunity to express my gratitude to everyone.

I am deeply indebted to my supervisor Dr. Steve Wilson under whom I started working on this project as well as I did my Postgraduate Honours. His help, stimulating suggestions and encouragement helped me in all the time of research and writing this thesis.

I would like to thank my co supervisor Dr. Cameron Spurr who kept an eye on the progress of my work and was always available when I needed his advice.

I would like to thank Mr. Peter Gore and Dr. David Boomsma (Seed Energy) for coming up with this project, financing it and supplying seeds for research. I would like to thank Dr. Dean Williams and Mr. John Juhasz for contributing valuable ideas in the beginning of this project.

I would like to thanks the University of Tasmania for supporting my studies and granting an International Postgraduate Research Scholarship.

I would like to acknowledge Dr. Sarah Salardini, Mr. Bill Peterson, Ms. Angela Richardson, Mr. Phil Andrews, Mr. Ian Cummings, Dr. Yuda Hariadi, Dr. Christiane Smethurst, Ms. Leng Cover, Ms. Ifa Ridwan, Ms. Jiayin Pang, Mr. Bhim Khatri, Mr. Sriram Padmanabhan, St. Michael's Collegiate Boarding House staff members and many others who directly or indirectly supported me at some point of my research.

My special gratitude towards my parents, my sister and brother in law and other members of my family for their loving support.

Abbreviation and Symbols

ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
ANOVA	Analysis of variance
ATP	Adenine tri phosphate
°C	degrees Celsius
Ca(NO ₃) ₂	Calcium nitrate
cm ³	Cubic Centimetre
CO ₂	Carbon dioxide
cm	Centimetre
DNA	Deoxyribonucleic acid
ETH	Ethanol
FR	Far red light
GA ₃	Gibberellic acid
g	Gram
h	Hours
ha	Hectare
IAA	Indole Acetic Acid
ISTA	International Seed Testing Association
KCl	Potassium Chloride
KIN	Kinetin
KNO ₃	Potassium nitrate
KOH	Potassium hydroxide
K ₃ PO ₄	Potassium phosphate
LSD	Least Significant Difference
m	Metre
mL	Milli Litre
mm	Milli Metre
mM	Milli Molar
MPa	Megapascal
mRNA	Messenger Ribonucleic acid

MSP	Mass Supplementary Pollination
NaNO ₃	Sodium nitrate
nm	Nano Metre
ODS	oleoyl phosphatidal choline desaturase
OSP	One Stop Pollination
P	Probability
PEG	Polyethylene glycol
Pfr	Far red absorbing (active) phytochrome
P/L	Private Limited
R	Red light
RNA	Ribonucleic acid
RWC	Relative water content
S	South
SEM	Standard Error of the Mean
SPSS	Statistical Package for the Social Science
STBA	Southern Tree Breeding Association Inc.
STDEV	Standard deviation
%	Percentage
®	Registered
μM	Micro Molar
μm	Micro Metre

Species Name

Common Names

Scientific Names

Allepo pine	<i>Pinus halepensis</i>
Alpine ash	<i>Eucalyptus delegatensis</i>
Alpine snowgum	<i>Eucalyptus nimphophila</i>
Ana tree	<i>Faidherbia albida</i>
Annual ragweed	<i>Ambrosia artemisiifolia</i>
Apple	<i>Malus domestica</i>
Areca palm	<i>Dyopsis lutescens</i>
Barley	<i>Hordeum</i> spp.
Beard-heath	<i>Leucopogon</i> spp.
Black box	<i>Eucalyptus largiflorens</i>
Blackbutts	<i>Eucalyptus pilularis</i>
Black ironbark	<i>Eucalyptus sideroxylon</i>
Black nightshade	<i>Solanum physalifolium</i>
Blue-white clover	<i>Trigonella coerulea</i>
Brindall berry/ Malabar Tamarind	<i>Garcinia gummi-gutta</i>
Broadleaf dock	<i>Rumex obtusifolius</i>
Broad leaf ironbark	<i>Eucalyptus fibrosa</i>
Broad-leaved peppermint	<i>Eucalyptus dives</i>
Brown barrel/ Cut tail	<i>Eucalyptus fastigata</i>
Bull mallee	<i>Eucalyptus behriana</i>
Bull thistle	<i>Cirsium vulgare</i> (Savi) Ten. }
Burning bush	<i>Dictamnus</i> spp.
Cabbage gum	<i>Eucalyptus pauciflora</i>
Calabrian pine	<i>Pinus brutia</i>
Capeweed	<i>Arctotheca calendula</i>
Celery	<i>Apium graveolens</i>
Charlock mustard	<i>Sinapsis arvensis</i>
Cloeziiana gum	<i>Eucalyptus cloeziana</i>

Cocklebur	<i>Xanthium strumarium</i>
Coolibah tree	<i>Eucalyptus microtheca</i>
Common elderberry	<i>Sambucus canadensis</i>
Common oat	<i>Avena sativa</i>
Common peppermint	<i>Eucalyptus radiata</i>
Corn/Shirley poppy	<i>Papaver rhoeas</i>
Cucumber	<i>Cucumis sativus</i>
Empress tree	<i>Paulownia tomentosa</i>
European beech	<i>Fagus sylvatica</i>
Evergreen clematis	<i>Clematis vitalba</i>
Forest redgum	<i>Eucalyptus tereticornis</i>
Giant-flowered phacelia	<i>Phacelia grandiflora</i>
Gippsland blue gum	<i>Eucalyptus globulus</i> subsp. <i>pseudoglobulus</i>
Good king Henry	<i>Chenopodium bonus-henricus</i>
Grevillea/ silky oak	<i>Grevillea robusta</i>
Grex box	<i>Eucalyptus moluccana</i>
Guinea flower	<i>Hibbertia amplexicaulis</i>
Gum cistus	<i>Cistus ladanifer</i>
Hedge mustard	<i>Sisymbrium officinale</i>
Iceberg/prickly lettuce	<i>Lactuca sativa</i>
Indonesian gum	<i>Eucalyptus deglupta</i>
Ivory lace	<i>Thryptomene calycina</i>
Jarrah/ Swan river mahogany	<i>Eucalyptus marginata</i>
Jointed rush	<i>Juncus articularis</i>
Knotweed	<i>Polygonum species</i>
Kybean mallee ash	<i>Eucalyptus kybeanensis</i>
Lacy scorpion weed	<i>Phacelia tanacetifolia</i>
Maize	<i>Zea mays</i>
Maritime pine	<i>Pinus pinaster</i>
Meadowfoam	<i>Limnanthes alba</i>
Mongolian Psammichloa	<i>Psammochloa villosa</i>

Mottlecah	<i>Eucalyptus macrocarpa</i>
Mountain ash	<i>Eucalyptus regnans</i>
Mount buffalo sallee/ gum	<i>Eucalyptus mitchelliana</i>
Mt. Wellington peppermint	<i>Eucalyptus coccifera</i>
Narrowleaf plantan	<i>Plantago lanceolata</i>
Northern bush honeysuckle	<i>Diervilla lonicera</i>
Norway maple	<i>Acer platanoides</i>
Oilseed rape	<i>Brassica napus</i>
Parodi Burkart	<i>Caesalpinia paraguariensis</i>
Peach	<i>Prunus persica</i>
Pennygrass	<i>Thlaspi arvense</i>
Pitcher plant	<i>Sarracenia</i> spp.
Poppy Anemone	<i>Anemone coronaria</i>
Proliferating bulrush	<i>Isolepis prolifera</i>
Purple coneflower/ Echinacea	<i>Echinacea purpurea</i>
Redroot pigweed	<i>Amaranthus retroflexus</i>
Red bloodwood	<i>Eucalyptus gummifera</i>
Red-capped gum	<i>Eucalyptus erythrocorys</i>
Red elderberry	<i>Sambucus pubens</i>
Red mallee	<i>Eucalyptus oleosa</i>
Red river gum	<i>Eucalyptus camaldulensis</i>
Red stringybark	<i>Eucalyptus macrorhyncha</i>
River peppermint	<i>Eucalyptus andreana</i>
Round leaf snow gum	<i>Eucalyptus perriniana</i>
Ryegrass	<i>Lolium perrene</i>
Salmon gum	<i>Eucalyptus salmonophloia</i>
Shining gum	<i>Eucalyptus nitens</i>
Silver maple	<i>Acer saccharinum</i>
Silver top ash	<i>Eucalyptus sieberi</i>
Small balsam	<i>Impatiens parviflora</i>
Sorghum	<i>Sorghum bicolor</i>
South American rush	<i>Juncus microcephalus</i>

Soybean	<i>Glycine max</i>
Spotted blue gum	<i>Eucalyptus globulus</i> subsp. <i>maidenii</i>
Spotted gum	<i>Eucalyptus maculata</i>
Spotted ladythumb	<i>Polygonum persicaria</i>
Sticky hopbush	<i>Dodonaea viscosa</i>
Striped maple	<i>Acer pensylvanicum</i>
Sugar maple	<i>Acer saccharum</i>
Swamp gum	<i>Eucalyptus ovata</i>
Swamp yate	<i>Eucalyptus occidentalis</i>
Swift parrots	<i>Lathamus discolor</i>
Sycamore leafed maple	<i>Acer pseudoplatanus</i>
Tartarian maple	<i>Acer tartaricum</i>
Tasmania blue gum	<i>Eucalyptus globulus</i> subsp. <i>globulus</i>
Tassel flower	<i>Amaranthus caudatus</i>
Thalla-cress	<i>Arabidopsis</i> spp.
Tomato	<i>Lycopersicon esculentum</i>
Tropical red box	<i>Eucalyptus brachyandra</i>
Triggerplants	<i>Stylidium affine</i>
	<i>Stylidium brunonianum</i>
Victoria blue gum	<i>Eucalyptus globulus</i> subsp. <i>bicostata</i>
Wheat	<i>Triticum aestivum</i>
Whispering bells	<i>Emmenanthe penduliflora</i>
White gum	<i>Eucalyptus wandoo</i>
Wild lettuce	<i>Lactuca serriola</i>
Wild oat	<i>Avena fatua</i>
Winter fat	<i>Eurotia lanata</i>
Yellow box	<i>Eucalyptus melliodora</i>
Yellow buttercup	<i>Hibbertia hypericoides</i>
Yellow horn poppy	<i>Glaucium flavum</i>
York gum	<i>Eucalyptus loxophleba</i>

ABSTRACT

Eucalyptus globulus is a major plantation forestry species in southern Australia. Commercial *E. globulus* seedlots have high viability (95%) and uniform germination, which is completed within 5-6 days. However commercial nurseries have reported delays in germination with seedling emergence spread over five to ten weeks. According to growers and seed suppliers the problem is not restricted to particular seedlots and occurs in most nurseries. Observations have suggested that a brief period of desiccation or high temperature between sowing and germination may lead to the delays, but no clear cause has been established. Further, there is nothing in the literature to indicate either cause or possible management strategies. According to the ISTA guidelines the germination period of *E. globulus* is 5-14 days under laboratory conditions and in the field, germination should occur before 26 days. The aim of the present project was to establish the factors responsible for this apparent induction of previously unreported secondary dormancy in *E. globulus* seed and to provide a basis for commercial management of the problem.

Initial laboratory trials concentrated on grower reports that short term desiccation in the first few days after sowing might be responsible. A wetting/drying cycle was found to cause an extended germination period. Initial imbibition in darkness for periods of 18 - 36 h, followed by air drying in open storage for 7 days at 25°C reduced subsequent germination in 12 light/ 12 dark photoperiod and slowed germination rate. Subsequent experiments demonstrated that this effect was largely regulated by light.

As a result of the initial results, further investigations into light sensitivity in *E. globulus* seeds were undertaken. Seeds initially imbibed in darkness for 18 - 36 h and then exposed to light showed decreased germination in darkness compared with seeds exposed throughout to continuous light, continuous dark or continuous light during imbibition followed by germination in darkness. These

results clearly indicate that initial exposure to darkness for 18 - 36 h followed by light caused a shift in germination behaviour.

Subsequent experiments on light quality showed that exposure to far red light throughout imbibition and germination substantially reduced germination percentage, whilst under some circumstances exposure to red light promoted germination. Such a model appears to explain the commercial problem, with the anomalous germination symptom induced by light conditions prevailing in the hour following sowing.

Results are discussed in terms of a possible physiological basis for the induction of an apparent light mediated secondary dormancy in this species. The practical implications for commercial nurseries are also considered, with particular emphasis on light management, rather than the initial ideas on water relations.